Physical interaction of RECQ5 helicase with RAD51 facilitates its anti-recombinase activity

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Abstract: Homologous recombination (HR) provides an efficient mechanism for error-free repair of DNA double-strand breaks (DSBs). However, HR can be also harmful as inappropriate or untimely HR events can give rise to lethal recombination intermediates and chromosome rearrangements. A critical step of HR is the formation of a RAD51 filament on single-stranded (ss)DNA, which mediates the invasion of a homologous DNA molecule. In mammalian cells, several DNA helicases have been implicated in the regulation of this process. RECQ5, a member of the RecQ family of DNA helicases, interacts physically with the RAD51 recombinase and disrupts RAD51 presynaptic filaments in a reaction dependent on ATP hydrolysis. Here, we have precisely mapped the RAD51-interacting domain of RECQ5 and generated mutants that fail to interact with RAD51. We show that although these mutants retain normal ATPase activity, they are impaired in their ability to displace RAD51 from ssDNA. Moreover, we show that ablation of RECQ5-RAD51 complex formation by a point mutation alleviates the inhibitory effect of RECQ5 on HR-mediated DSB repair. These findings provide support for the proposal that interaction with RAD51 is critical for the anti-recombinase attribute of RECQ5.

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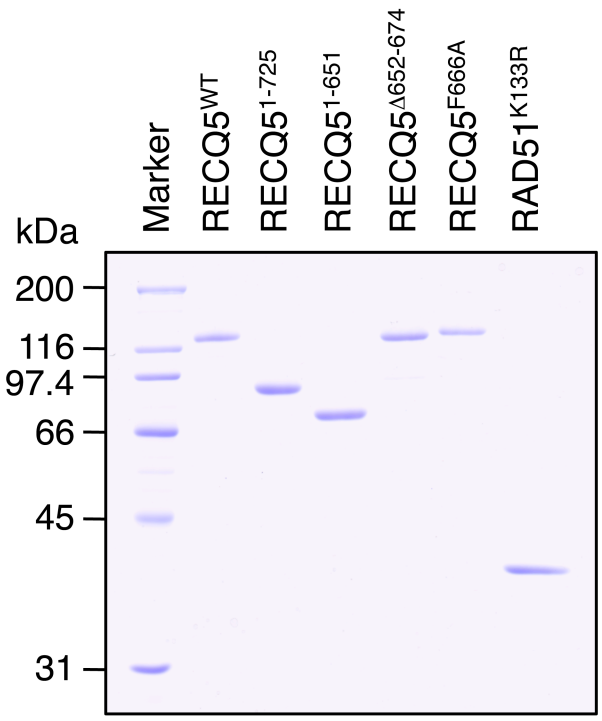

FIGURE S1. **SDS PAGE analysis of purified proteins used in this study.** Gel was stained with Coomassie Brilliant Blue R250.

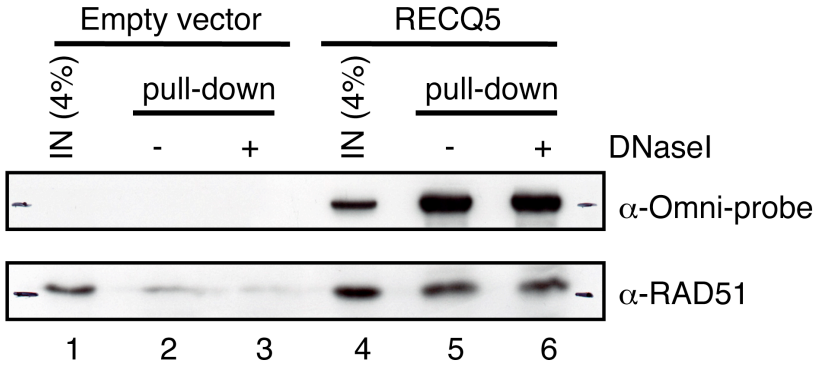

FIGURE S2. **Interaction between RECQ5 and RAD51 *in vivo* is not mediated through DNA.** 293T cells were transfected with a vector for $(His)_6$-Xpress tagged RECQ5 or with the empty vector. Cell extracts (800 μg of protein) were incubated with or without DNase I (20 U) at 25°C for 30 minutes before addition of Ni-NTA beads (25 μl). Bound proteins were separated by SDS-PAGE and analyzed by Western blotting using Omni-probe (*upper panel*; $(His)_6$-Xpress-RECQ5) and anti-RAD51 antibodies (*lower panel*). IN, Input.

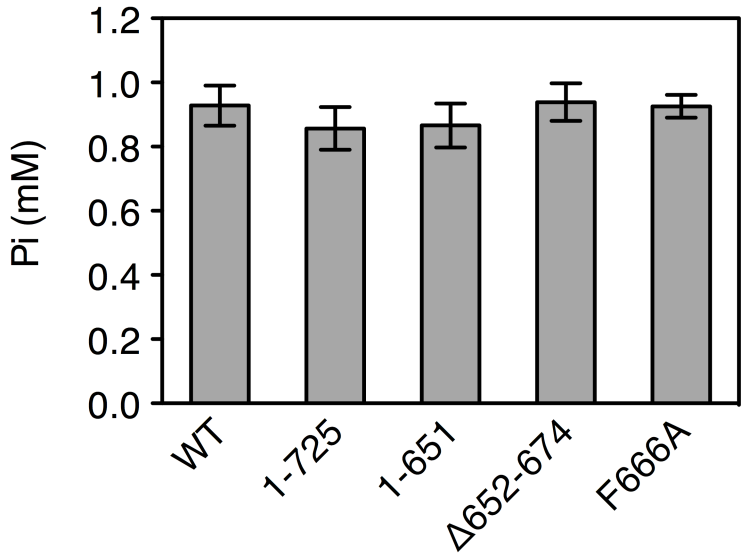

FIGURE S3. **ATPase activitity of RECQ5 mutants.** ATPase activity of RECQ5 variants was determined by colorimetric estimation of the amount of inorganic phosphate (P_i) released by ATP hydrolysis. Reactions were carried out in buffer R at 37°C for 30 minutes and contained 20 nM RECQ5, 25 μg/ml M13ssDNA and 2 mM ATP. Reactions were stopped by addition of EDTA and the amount of inorganic phosphate (P_i) was determined by malachite green assay. Briefly, 10 μl of terminated reactions were added to 30 μl of 0.1 M EDTA (pH 8) in a 96-well microplate followed by addition of 100 μl of a freshly prepared 3:1 mixture of water / 5.72% (w/v) ammonium molybdate in 6 M HCl and 50 μl of 0.0812% (w/v) malachite green in water. The mixtures were incubated for five minutes before measurement of absorbance at 620 nm in a SpectraMax M5 reader (Molecular Devices). The concentration of P_i released by ATP hydrolysis was determined from a calibration curve derived from solutions of known P_i concentration (KH_2PO_4).